INTERNATIONAL SEARCH REPORT

09/857129

International Application No
PCT/GB 99/03973

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 A61P43/00 A61K31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	COOPER DN ET AL: "Inherited Factor X deficiency: Molecular genetics and pathophysiology" THROMBOSIS AND HAEMOSTASIS, vol. 78, no. 1, July 1997 (1997-07), pages 161-172, XP000890130 page 166; table 1 —	1-5,7-11
A	MIYATA T ET AL: "Factor X Nagoya 1 and Nagoya 2: a CRM- defiency and a dysfunctional CRM+ Factor X defiency characterized by substitution of Arg306 by Cys and of Gly366 by Ser, respectively." THROMBOSIS AND HAEMOSTASIS, vol. 79, no. 3, March 1998 (1998-03), pages 486-90, XP000889942 the whole document —	1-5,7-11

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 March 2000	21/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax (+31–70) 340–3016	Osborne, H

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03973

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCHAFER AJ ET AL: "DNA variation and the future of human genetics" NATURE BIOLOGY, vol. 16, January 1998 (1998-01), XP000890128 the whole document	1
A	WO 98 38318 A (FALKNER FALKO GUENTER ;HIMMELSPACH MICHELE (AT); EIBL JOHANN (AT);) 3 September 1998 (1998-09-03) see SEQ ID No 43, where in bp position 793, an A is indicated in place of a C found in EMBL ACC No L00396, corresponding to nucleic acid sequence of Exon 7.	1,2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/03973

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 10 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

[redacted]ormation on patent family members

[redacted]nal Application No
PCT/GB 99/03973

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9838318 A	03-09-1998	AT 405517 B	27-09-1999
		AT 33697 A	15-01-1999
		AU 6080898 A	18-09-1998
		NO 994136 A	27-10-1999

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* PHM 70433/WO

Box No. I TITLE OF INVENTION

CHEMICAL COMPOUNDS

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ZENECA Limited
15 Stanhope Gate
LONDON
W1Y 6LN
GB

☐ This person is also inventor.

Telephone No.
01625 516573

Facsimile No.
01625 583358

Teleprinter No.
669095/669388 ZENPHA G

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ANAND, Rakesh
Alderley Park
Macclesfield
Cheshire
GB-SK10 4TG
GB

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is,country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BILL, Kevin
ASTRAZENECA PLC
Global Intellectual Property, Patents.
Mereside, Alderley Park
Macclesfield, Cheshire.
SK10 4TG. GB.

Telephone No.
01625 512461

Facsimile No.
01625 583358

Teleprinter No.
669095/669388 ZENPHA G

☐ **Adress for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III **FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS**

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MORTEN, John Edward Norris
Alderley Park
Macclesfield
Cheshire
GB-SK10 4TG
GB

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SMITH, John Craig
Alderley Park
Macclesfield
Cheshire
GB-SK10 4TG
GB

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- [X] AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- [X] EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [X] OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] AL Albania .....
- [X] AM Armenia .....
- [X] AT Austria .....
- [X] AU Australia .....
- [X] AZ Azerbaijan
- [X] BA Bosnia and Herzegovina .....
- [X] BB Barbados
- [X] BG Bulgaria .....
- [X] BR Brazil .....
- [X] BY Belarus .....
- [X] CA Canada
- [X] CH and LI Switzerland and Liechtenstein
- [X] CN China .....
- [X] CU Cuba .....
- [X] CZ Czech Republic .....
- [X] DE Germany .....
- [X] DK Denmark .....
- [X] EE Estonia .....
- [X] ES Spain .....
- [X] FI Finland .....
- [X] GB United Kingdom
- [X] GD Grenada
- [X] GE Georgia .....
- [X] GH Ghana .....
- [X] GM Gambia
- [X] HR Croatia .....
- [X] HU Hungary .....
- [X] ID Indonesia
- [X] IL Israel .....
- [X] IN India .....
- [X] IS Iceland
- [X] JP Japan .....
- [X] KE Kenya .....
- [X] KG Kyrgyzstan .....
- [X] KP Democratic People's Republic of Korea .....
- [X] KR Republic of Korea .....
- [X] KZ Kazakhstan .....
- [X] LC Saint Lucia
- [X] LK Sri Lanka
- [X] LR Liberia
- [X] LS Lesotho .....
- [X] LT Lithuania
- [X] LU Luxembourg
- [X] LV Latvia
- [X] MD Republic of Moldova .....
- [X] MG Madagascar .....
- [X] MK The former Yugoslav Republic of Macedonia .....
- [X] MN Mongolia
- [X] MW Malawi .....
- [X] MX Mexico .....
- [X] NO Norway
- [X] NZ New Zealand .....
- [X] PL Poland .....
- [X] PT Portugal .....
- [X] RO Romania
- [X] RU Russian Federation .....
- [X] SD Sudan
- [X] SE Sweden
- [X] SG Singapore
- [X] SI Slovenia .....
- [X] SK Slovakia .....
- [X] SL Sierra Leone
- [X] TJ Tajikistan .....
- [X] TM Turkmenistan .....
- [X] TR Turkey .....
- [X] TT Trinidad and Tobago .....
- [X] UA Ukraine .....
- [X] UG Uganda .....
- [X] US United States of America .....
- [X] UZ Uzbekistan .....
- [X] VN Viet Nam .....
- [X] YU Yugoslavia .....
- [X] ZW Zimbabwe .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- [X] DM - DOMINICA
- [X] ZA - SOUTH AFRICA
- [X] UE - UNITED ARAB EMIRATES
- [X] CR - COSTA RICA
- [X] MA - MOROCCO

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority clai[redacted] indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Offic
item (1) 05-Dec-98 (05.12.98)	9826747.9	GB		
item (2)				
item (3)				

[X] The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): Item (1)

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA /

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	4
description (excluding sequence listing part)	:	16
claims	:	2
abstract	:	1
drawings	:	
sequence listing part of description	:	3
Total number of sheets	:	26

This international application is **accompanied by** the item(s) marked below:

1. [X] fee calculation sheet
2. [X] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [X] priority document(s) identified in Box No. VI as item(s):
6. [ ] translation of international application into (language):
7. [ ] separate indications concerning deposited microorganism or other biological material
8. [X] nucleotide and/or amino acid sequence listing in computer readable form
9. [ ] other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Kevin BILL
AGENT FOR APPLICANT

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY /857129

PCT

From the INTERNATIONAL BUREAU

To:

BILL, Kevin
Global Intellectual Property
AstraZeneca UK Limited
Mereside
Alderley Park
Macclesfield, Cheshire SK10 4TG
ROYAUME-UNI

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year) 08 May 2000 (08.05.00)	
Applicant's or agent's file reference PHM 70433/WO	**IMPORTANT NOTIFICATION**
International application No. PCT/GB99/03973	**International filing date** (day/month/year) 30 November 1999 (30.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

ZENECA LIMITED
15 Stanhope Gate
London W1Y 6LN
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address

ASTRAZENECA UK LIMITED
15 Stanhope Gate
London W1Y 6LN
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer

Céline Faust

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

09/857129

Copy - DMT

PCT

From the INTERNATIONAL BUREAU

RECEIVED
27 SEP 2000
ASTRA ZENECA
GLOBAL INTELLECTUAL PROPERTY

To:

BILL, Kevin
Global Intellectual Property
AstraZeneca
P.O. Box 272, Mereside
Alderley Park
Macclesfield, Cheshire SK10 4TG
ROYAUME-UNI

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year) 18 September 2000 (18.09.00)	
Applicant's or agent's file reference PHM 70433/WO	**IMPORTANT NOTIFICATION**
International application No. PCT/GB99/03973	**International filing date** (day/month/year) 30 November 1999 (30.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
ASTRAZENECA UK LIMITED 15 Stanhope Gate London W1Y 6LN United Kingdom	GB	GB
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
ASTRAZENECA AB S-151 85 Södertälje Sweden	SE	SE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer C. CARRIÉ Christine Carrié
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

003530710

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PHM.70433/WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/03973	30/11/1999	03/12/1998
International Patent Classification (IPC) or national classification and IPC		
C12Q1/68		
Applicant		
ASTRAZENECA AB et al.		

CODE | DATE | NTD

REC'D 06 FEB 2001 GIPS

DATA ENTERED DR SLC

FINAL CHECK

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/06/2000	01.02.2001
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Tilkorn, A-C Telephone No. +49 89 2399 8688

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/03973

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-16 as originally filed

Claims, No.:

1-12 as originally filed

Sequence listing part of the description, pages:

1-3, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:
   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 10, 12 with respect to industrial applicability.

because:

   ☒ the said international application, or the said claims Nos. 10-12 with respect to industrial applicability relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9,12
	No:	Claims	10,11
Inventive step (IS)	Yes:	Claims	-

	No:	Claims	1-12
Industrial applicability (IA)	Yes:	Claims	1-9,11
	No:	Claims	-

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

This written opinion also takes into consideration pages 1-3 of the Sequence Listing (i.e information concerning SEQ ID NOs 1-6).

Re Item III

Claims 10 relates to medical uses considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT.

In turn **claim 12** relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(v) PCT since it amounts to mere presentation of information (Guidelines IV-2.4(e)).

Consequently, no opinion is formulated with respect to the industrial applicability of **claims 10** and **12** (Art 34(4)(a)(i) PCT).

Re Item V

The following documents are referred to in this communication:

D1: THROMBOSIS AND HAEMOSTASIS, vol. 78, no. 1, July 1997 (1997-07), pages 161-172
D2 WO 98 21188

1 Novelty (Art 33(2) PCT):

1.1 **Claim 11** does not satisfy Art 33(2) PCT, because the use of Factor Xa ligand antagonist drugs for treating Factor Xa and/or Factor X mediated disease in a human is known from each of the documents D1 and D2, which are cited in the present application (appl: p 11 l 20-21). The expression "in a human diagnosed as having a single nucleotide polymorphism at position 41 in exon 5 [...] and/or at position 57 in exon 7 in the Factor X gene..." does not specify an allele and does therefore not restrict the treatment to individuals carrying a certain allele of each polymorphic site (see also item VIII below). For the same reasons, **claim 10** that

relates to a method of treatment is not novel, either.

1.2 **Claim 1** is novel, because the polymorphisms at position 41 in exon 5 and at position 57 in exon 7 have previously not been disclosed. Consequently, also **claims 2-5** are novel.

1.3 **Claim 6** is novel, because none of the available documents discloses either of the alleles of claim 6. **Claim 12** is novel, accordingly. Similarly, the allele-specific primers and probes as well as the diagnostic kit are novel (**claims 7-9**).

2 Inventive Step (Art 33(3) PCT):

Claim 1 does not appear to satisfy Art 33(3) PCT for the following reasons:
D1, which is considered to represent the closest prior art, discloses Factor X deficiencies. Claim 1 is distinguished from the disclosure of D1 in that D1 does not disclose the specific polymorphisms.
However, the discovery of the polymorphisms of claim 1 does apparently not solve a problem, since said polymorphisms are not characterized in the present disclosure as being linked to a certain disease or condition.
The same argument applies to **claims 2-9** and **12**. The diseases that are mentioned in the application (p 11 l 6 ff) are not linked to the specific polymorphisms of the invention. Moreover, there is no technical indicator in the application that the discovered polymorphisms increase the probability of pathological conditions (appl. p 2 l 4-6). In relation to this deficiency see also point 2 of item VIII below.

3 Industrial Applicability (Art 33(4) PCT):

For the assessment of **claims 10** and **11** on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII

- The application does not explicitly disclose a specific Factor Xa or Factor X inhibitor. However, reference is made to published documents, e.g. D2 (appl.: p 11 l 21) which disclose specific Factor Xa inhibitors. In order for the application to be self-contained at least one specific Factor Xa inhibitor should have been incorporated in the description (Guidelines II 4.17).

- The expression "incorporated herein by reference" in respect of prior art documents (e.g. page 8 l 22-23) leads to a doubt as to whether the requirement of the description being self-contained is satisfied (Guidelines II 4.17).

Re Item VIII

1 The reference to database accession numbers in the claims (**claims 1,6-8,10,11**) does not comply with the requirement of clarity (Art 6 PCT) because database entries can be modified after the original submission. Thus, the sequences referred to in the claims are not clearly and unambiguously defined by database accession numbers.

2 The discovered polymorphisms in the genes of Factor X and/or Factor Xa are meant to be relevant for Factor X and/or Factor Xa mediated disease, but the application lacks experimental evidence and therefore technical support (Guidelines III 6.3). The relevance of the polymorphisms for thrombotic disease (appl.: p 6 l 20, 32) appears to be speculative.

3 **Claims 7-9** do not meet the requirements of Art 6 PCT. The expressions "primer" and "oligonucleotide probes" employed in these claims do not restrict the length of the nucleic acid sequences therein referred to. Consequently, the scope of said claims is rendered unclear. To comply with the requirement of clarity (Art 6 PCT), the length of the primer/probes as set forth in the description (p 9 l 17-18; p 10 l 3-4) should have been incorporated into the claims.

4 **Claim 10** does not satisfy Art 6 PCT because the expression "a human in need" is vague and renders the scope of the claim unclear.

5 **Claims 10** and **11** do not comply with Art 6 PCT, because the expression "Factor Xa ligand antagonist drug" is not clear. There is no mention of any Factor Xa ligand throughout the application and the state of the art does not disclose such a ligand either. From the description it is understood that Factor Xa and/or Factor X inhibitors are meant (appl.: p 11 l 4-5 and l 20). In order to clarify the claims, the objected formulation could have been replaced by "Factor Xa and/or Factor X inhibitor".

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PHM.70433/WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/03973	30/11/1999	05/12/1998
International Patent Classification (IPC) or national classification and IPC		
C12Q1/68		
Applicant		
ASTRAZENECA AB et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/06/2000	01.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Tilkorn, A-C Telephone No. +49 89 2399 8688

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/03973

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-16 as originally filed

Claims, No.:

1-12 as originally filed

Sequence listing part of the description, pages:

1-3, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:
   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 10, 12 with respect to industrial applicability.

because:

   ☒ the said international application, or the said claims Nos. 10-12 with respect to industrial applicability relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9,12
	No:	Claims	10,11
Inventive step (IS)	Yes:	Claims	-

	No:	Claims	1-12
Industrial applicability (IA)	Yes:	Claims	1-9,11
	No:	Claims	-

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

This written opinion also takes into consideration pages 1-3 of the Sequence Listing (i.e information concerning SEQ ID NOs 1-6).

Re Item III

Claims 10 relates to medical uses considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT.

In turn **claim 12** relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(v) PCT since it amounts to mere presentation of information (Guidelines IV-2.4(e)).

Consequently, no opinion is formulated with respect to the industrial applicability of **claims 10** and **12** (Art 34(4)(a)(i) PCT).

Re Item V

The following documents are referred to in this communication:

D1: THROMBOSIS AND HAEMOSTASIS, vol. 78, no. 1, July 1997 (1997-07), pages 161-172
D2 WO 98 21188

1 Novelty (Art 33(2) PCT):

1.1 **Claim 11** does not satisfy Art 33(2) PCT, because the use of Factor Xa ligand antagonist drugs for treating Factor Xa and/or Factor X mediated disease in a human is known from each of the documents D1 and D2, which are cited in the present application (appl: p 11 l 20-21). The expression "in a human diagnosed as having a single nucleotide polymorphism at position 41 in exon 5 [...] and/or at position 57 in exon 7 in the Factor X gene..." does not specify an allele and does therefore not restrict the treatment to individuals carrying a certain allele of each polymorphic site (see also item VIII below). For the same reasons, **claim 10** that

relates to a method of treatment is not novel, either.

1.2 **Claim 1** is novel, because the polymorphisms at position 41 in exon 5 and at position 57 in exon 7 have previously not been disclosed. Consequently, also **claims 2-5** are novel.

1.3 **Claim 6** is novel, because none of the available documents discloses either of the alleles of claim 6. **Claim 12** is novel, accordingly. Similarly, the allele-specific primers and probes as well as the diagnostic kit are novel (**claims 7-9**).

2 Inventive Step (Art 33(3) PCT):

Claim 1 does not appear to satisfy Art 33(3) PCT for the following reasons:
D1, which is considered to represent the closest prior art, discloses Factor X deficiencies. Claim 1 is distinguished from the disclosure of D1 in that D1 does not disclose the specific polymorphisms.
However, the discovery of the polymorphisms of claim 1 does apparently not solve a problem, since said polymorphisms are not characterized in the present disclosure as being linked to a certain disease or condition.
The same argument applies to **claims 2-9** and **12**. The diseases that are mentioned in the application (p 11 l 6 ff) are not linked to the specific polymorphisms of the invention. Moreover, there is no technical indicator in the application that the discovered polymorphisms increase the probability of pathological conditions (appl. p 2 l 4-6). In relation to this deficiency see also point 2 of item VIII below.

3 Industrial Applicability (Art 33(4) PCT):

For the assessment of **claims 10** and **11** on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII

- The application does not explicitly disclose a specific Factor Xa or Factor X inhibitor. However, reference is made to published documents, e.g. D2 (appl.: p 11 l 21) which disclose specific Factor Xa inhibitors. In order for the application to be self-contained at least one specific Factor Xa inhibitor should have been incorporated in the description (Guidelines II 4.17).

- The expression "incorporated herein by reference" in respect of prior art documents (e.g. page 8 l 22-23) leads to a doubt as to whether the requirement of the description being self-contained is satisfied (Guidelines II 4.17).

Re Item VIII

1 The reference to database accession numbers in the claims (**claims 1,6-8,10,11**) does not comply with the requirement of clarity (Art 6 PCT) because database entries can be modified after the original submission. Thus, the sequences referred to in the claims are not clearly and unambiguously defined by database accession numbers.

2 The discovered polymorphisms in the genes of Factor X and/or Factor Xa are meant to be relevant for Factor X and/or Factor Xa mediated disease, but the application lacks experimental evidence and therefore technical support (Guidelines III 6.3). The relevance of the polymorphisms for thrombotic disease (appl.: p 6 l 20, 32) appears to be speculative.

3 **Claims 7-9** do not meet the requirements of Art 6 PCT. The expressions "primer" and "oligonucleotide probes" employed in these claims do not restrict the length of the nucleic acid sequences therein referred to. Consequently, the scope of said claims is rendered unclear. To comply with the requirement of clarity (Art 6 PCT), the length of the primer/probes as set forth in the description (p 9 l 17-18; p 10 l 3-4) should have been incorporated into the claims.

4 **Claim 10** does not satisfy Art 6 PCT because the expression "a human in need" is vague and renders the scope of the claim unclear.

5 **Claims 10** and **11** do not comply with Art 6 PCT, because the expression "Factor Xa ligand antagonist drug" is not clear. There is no mention of any Factor Xa ligand throughout the application and the state of the art does not disclose such a ligand either. From the description it is understood that Factor Xa and/or Factor X inhibitors are meant (appl.: p 11 l 4-5 and l 20). In order to clarify the claims, the objected formulation could have been replaced by "Factor Xa and/or Factor X inhibitor".

PATENT COOPERATION TREATY

PCT

09/857129

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
PHM 70433/WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/03973	30/11/1999	05/12/1998
Applicant		
ZENECA LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

   USE OF FACTOR X POLYMORPHISM IN THE DIAGNOSIS AND TREATMENT OF FACTOR X AND/OR FACTOR XA MEDIATED DISEAES

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. --

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/03973

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 10 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]GB 99/03973

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 A61P43/00 A61K31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	COOPER DN ET AL: "Inherited Factor X deficiency: Molecular genetics and pathophysiology" THROMBOSIS AND HAEMOSTASIS, vol. 78, no. 1, July 1997 (1997-07), pages 161-172, XP000890130 page 166; table 1 ---	1-5,7-11
A	MIYATA T ET AL: "Factor X Nagoya 1 and Nagoya 2: a CRM- defiency and a dysfunctional CRM+ Factor X defiency characterized by substitution of Arg306 by Cys and of Gly366 by Ser, respectively." THROMBOSIS AND HAEMOSTASIS, vol. 79, no. 3, March 1998 (1998-03), pages 486-90, XP000889942 the whole document ---	1-5,7-11

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 March 2000	21/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Osborne, H

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]GB 99/03973

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	SCHAFER AJ ET AL: "DNA variation and the future of human genetics" NATURE BIOLOGY, vol. 16, January 1998 (1998-01), XP000890128 the whole document ---	1
A	WO 98 38318 A (FALKNER FALKO GUENTER ;HIMMELSPACH MICHELE (AT); EIBL JOHANN (AT);) 3 September 1998 (1998-09-03) see SEQ ID No 43, where in bp position 793, an A is indicated in place of a C found in EMBL ACC No L00396, corresponding to nucleic acid sequence of Exon 7. -----	1,2

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
GB 99/03973

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9838318 A	03-09-1998	AT 405517 B	27-09-1999
		AT 33697 A	15-01-1999
		AU 6080898 A	18-09-1998
		NO 994136 A	27-10-1999

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 07 August 2000 (07.08.00)	
International application No. PCT/GB99/03973	**Applicant's or agent's file reference** PHM 70433/WO
International filing date (day/month/year) 30 November 1999 (30.11.99)	**Priority date** (day/month/year) 05 December 1998 (05.12.98)
Applicant ANAND, Rakesh et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

20 June 2000 (20.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Pascal Piriou
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Copy for the Elected Office (EO/US)

PCT/GB99/03973

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

BILL, Kevin
Global Intellectual Property
AstraZeneca
P.O. Box 272, Mereside
Alderley Park
Macclesfield, Cheshire SK10 4TG
ROYAUME-UNI

Date of mailing (day/month/year)
18 September 2000 (18.09.00)

Applicant's or agent's file reference
PHM 70433/WO

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/03973

International filing date (day/month/year)
30 November 1999 (30.11.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
ASTRAZENECA UK LIMITED
15 Stanhope Gate
London W1Y 6LN
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
ASTRAZENECA AB
S-151 85 Södertälje
Sweden

State of Nationality: SE
State of Residence: SE
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
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003530710

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NOTIFICATION OF THE RECORDING OF A CHANGE

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To:

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Date of mailing (day/month/year)
18 September 2000 (18.09.00)

Applicant's or agent's file reference
PHM 70433/WO

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/03973

International filing date (day/month/year)
30 November 1999 (30.11.99)

1. The following indications appeared on record concerning:

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3. Further observations, if necessary:

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Form PCT/IB/306 (March 1994)

003530499

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12Q 1/68, A61P 43/00, A61K 31/00

A1

(11) International Publication Number: WO 00/34515

(43) International Publication Date: 15 June 2000 (15.06.00)

(21) International Application Number: PCT/GB99/03973

(22) International Filing Date: 30 November 1999 (30.11.99)

(30) Priority Data:
9826747.9 5 December 1998 (05.12.98) GB

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: USE OF FACTOR X POLYMORPHISM IN THE DIAGNOSIS AND TREATMENT OF FACTOR X AND/OR FACTOR XA MEDIATED DISEASES

(57) Abstract

This invention relates to polymorphisms in the human Factor X gene, in particular to the discovery of two single nucleotide polymorphisms in the coding sequence of the human Factor X gene. The invention also relates to methods and materials for analysing allelic variation in the Factor X gene, and to the use of Factor X polymorphism in the diagnosis and treatment of Factor X and/or Factor Xa–mediated diseases, such as thrombotic diseases.

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USE OF FACTOR X POLYMORPHISM IN THE DIAGNOSIS AND TREATMENT OF FACTOR X AND/OR FACTOR XA MEDIATED DISEASES

This invention relates to polymorphisms in the Factor X gene. The invention also relates to methods and materials for analysing allelic variation in the Factor X gene, and to the use of Factor X polymorphism in the diagnosis and treatment of Factor X and/or Factor Xa-mediated diseases, such as thrombotic diseases.

Factor Xa is one of a cascade of proteases involved in the complex process of blood coagulation. The protease known as thrombin is the final protease in the cascade and Factor Xa is the preceding protease which cleaves prothrombin to generate thrombin. Factor Xa is produced by cleavage of the zymogen precursor Factor X, by activated factor VII. For a review of the process of blood coagulation see Rock and Wells (1997) Crit Rev Clin Lab Sci **34**, 475-501 and for a review of the Biochemistry of Factor X see Hertzberg (1994) Blood Reviews 8, 56-62.

Certain compounds are known to possess Factor Xa inhibitory properties and the field has been reviewed by R.B. Wallis, Current Opinion in Therapeutic Patents, 1993, 1173-1179 and Yamazaki (1995) Drugs of the Future 20, 911-918. Thus it is known that two proteins, one known as antistatin and the other known as tick anticoagulant protein (TAP), are specific Factor Xa inhibitors which possess antithrombotic properties in various animal models of thrombotic disease.

It is also known that certain non-peptidic compounds possess Factor Xa inhibitory properties. Of the low molecular weight inhibitors mentioned in the review by R.B. Wallis, all possessed a strongly basic group such as an amidinophenyl or amidinonaphthyl group.

The sequence of Factor X was published by Leytus et al (1986) Biochemistry **25**, 5098-5102. The sequence was submitted to the EMBL database as separate exons: Exon 1 (EMBL Accession Number -L00390), Exon 2 (EMBL Accession Number - L00391), Exon 3 (EMBL Accession Number - L00392), Exon 4 (EMBL Accession Number - L00393), Exon 5 (EMBL Accession Number - L00394), Exon 6 ((EMBL Accession Number - L00395), Exon 7 (EMBL Accession Number - L00396), and Exon 8 (EMBL Accession Number - L29433). All positions herein relate to the position in the appropriate EMBL Accession number unless stated otherwise or apparent from the context.

Mutations in the Factor X gene which lead to Factor X deficiency and a clinical phenotype are well documented (For a review of Factor X mutations and Factor X deficiency see Cooper et al (1997) Thrombosis and Haemostasis 78, 161-172).

Other variation in DNA sequence (polymorphisms) may not lead to Factor X deficiency but may increase the probability of pathological conditions or affect drug response or may be genetically linked to other polymorphisms which do so.

One approach is to use knowledge of polymorphisms to help identify patients most suited to therapy with particular pharmaceutical agents (this is often termed "pharmacogenetics") . Pharmacogenetics can also be used in pharmaceutical research to assist the drug selection process. Polymorphisms are used in mapping the human genome and to elucidate the genetic component of diseases. The reader is directed to the following references for background details on pharmacogenetics and other uses of polymorphism detection: Linder *et al.* (1997), Clinical Chemistry, **43**, 254; Marshall (1997), Nature Biotechnology, **15**, 1249; International Patent Application WO 97/40462, Spectra Biomedical; and Schafer *et al.* (1998), Nature Biotechnology, **16**, 33.

Clinical trials have shown that patient response to treatment with pharmaceuticals is often heterogeneous. Thus there is a need for improved approaches to pharmaceutical agent design and therapy.

The present invention is based on the discovery of two single nucleotide polymorphisms (SNPs) in the coding sequence of the human Factor X gene.

According to one aspect of the present invention there is provided a method for the diagnosis of a single nucleotide polymorphism in a Factor X gene in a human, which method comprises determining the sequence of the nucleic acid of the human

at position 41 in exon 5 of the Factor X gene as defined by the position in EMBL ACCESSION NO. L00394, and/or

at position 57 in exon 7 of the Factor X gene as defined by the position in EMBL ACCESSION NO. L00396 and determining the status of the human by reference to polymorphism in the Factor X gene.

According to another aspect of the present invention there is provided a method for the diagnosis of a single nucleotide polymorphism in a Factor X gene in a human, which method comprises determining the sequence of the nucleic acid of the human

at position 41 in exon 5 of the Factor X gene as defined by the position in EMBL ACCESSION NO. L00394, and/or

at position 57 in exon 7 of the Factor X gene as defined by the position in EMBL ACCESSION NO. L00396 and determining the status of the human by reference to polymorphism in the Factor X gene.

The term human includes both a human having or suspected of having a Factor X-mediated disease and an asymptomatic human who may be tested for predisposition or susceptibility to such disease. At each position the human may be homozygous for an allele or the human may be a heterozygote.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at exon 5 position 41 is presence of C and/or T.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at exon 7 position 57 is presence of C and/or T.

Subsequently to the present invention, Cargill et al have confirmed the presence of a single nucleotide polymorphism in human Factor X at exon 5 position 41 and/or at exon 7 position 57 (Cargill et al., Nature Genetics, 22, 231-239, 1999).

The method for diagnosis is preferably one in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length polymorphism.

In another aspect of the invention we provide a method for the diagnosis of Factor X- and/or Factor Xa-mediated disease, which method comprises:

i) obtaining sample nucleic acid from an individual,

ii) detecting the presence or absence of a variant nucleotide at position 41 in exon 5 of the Factor X gene as defined by the position in EMBL ACCESSION NO. L00394, and/or

at position 57 in exon 7 of the Factor X gene as defined by the position in EMBL ACCESSION NO. L00396,

iii) determining the status of the individual by reference to polymorphism in the Factor X gene.

Allelic variation at exon 5 position 41 consists of a single base substitution from C (the published base), preferably to T. Allelic variation at exon 7 position 57 consists of a single base substitution from C (the published base), preferably to T.

The status of the individual may be determined by reference to allelic variation at any one or both positions optionally in combination with any other polymorphism that is or becomes known.

The test sample of nucleic acid is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before analysis of allelic variation.

It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system. Table 1 lists a number of mutation detection techniques, some based on the PCR. These may be used in combination with a number of signal generation systems, a selection of which is listed in Table 2. Further amplification techniques are listed in Table 3. Many current methods for the detection of allelic variation are reviewed by Nollau *et al.*, Clin. Chem. **43**, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

Abbreviations:

ALEX™	Amplification refractory mutation system linear extension
APEX	Arrayed primer extension
ARMS™	Amplification refractory mutation system
b-DNA	Branched DNA
CMC	Chemical mismatch cleavage
bp	base pair

COPS	Competitive oligonucleotide priming system
DGGE	Denaturing gradient gel electrophoresis
FRET	Fluorescence resonance energy transfer
LCR	Ligase chain reaction
MASDA	Multiple allele specific diagnostic assay
NASBA	Nucleic acid sequence based amplification
OLA	Oligonucleotide ligation assay
PCR	Polymerase chain reaction
PTT	Protein truncation test
RFLP	Restriction fragment length polymorphism
SDA	Strand displacement amplification
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism analysis
SSR	Self sustained replication
TGGE	Temperature gradient gel electrophoresis

Table 1 - Mutation Detection Techniques

General: DNA sequencing, Sequencing by hybridisation

Scanning: PTT*, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage

* Note: not useful for detection of promoter polymorphisms.

Hybridisation Based

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips)

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, **14**, 303; WO 95/13399 (Public Health Inst., New York)

Extension Based: ARMS™, ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, **17**, 2347.

Incorporation Based: Mini-sequencing, APEX

Restriction Enzyme Based: RFLP, Restriction site generating PCR

Ligation Based: OLA

Other: Invader assay

Table 2 - Signal Generation or Detection Systems

Fluorescence: FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)

Other: Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric, Hybridisation protection assay, Mass spectrometry

Table 3 - Further Amplification Methods

SSR, NASBA, LCR, SDA, b-DNA

Preferred mutation detection techniques include ARMS™, ALEX™, COPS, Taqman, Molecular Beacons, RFLP, and restriction site based PCR and FRET techniques.

Particularly preferred methods include ARMS™ and RFLP based methods. ARMS™ is an especially preferred method.

In a further aspect, the diagnostic methods of the invention are used to assess the efficacy of therapeutic compounds in the treatment of Factor X and/or Factor Xa-mediated diseases, such as thrombotic diseases.

Assays, for example reporter-based assays, may be devised to detect whether one or more of the above polymorphisms affect transcription levels and/or message stability.

Individuals who carry particular allelic variants of the Factor X gene may therefore exhibit differences in their ability to regulate protein biosynthesis under different physiological conditions and will display altered abilities to react to different diseases. In addition, differences in protein regulation arising as a result of allelic variation may have a direct effect on the response of an individual to drug therapy. The diagnostic methods of the invention may be useful both to predict the clinical response to such agents and to determine therapeutic dose.

In a further aspect, the diagnostic methods of the invention, are used to assess the predisposition of an individual to diseases mediated by Factor X and/or Factor Xa. This may be particularly relevant in the development of thrombotic disease and other diseases which are

modulated by Factor X and/or Factor Xa. The present invention may be used to recognise individuals who are particularly at risk from developing these conditions.

Low frequency polymorphisms may be particularly useful for haplotyping as described below. A haplotype is a set of alleles found at linked polymorphic sites (such as within a gene) on a single (paternal or maternal) chromosome. If recombination within the gene is random, there may be as many as 2^n haplotypes, where 2 is the number of alleles at each SNP and n is the number of SNPs. One approach to identifying mutations or polymorphisms which are correlated with clinical response is to carry out an association study using all the haplotypes that can be identified in the population of interest. The frequency of each haplotype is limited by the frequency of its rarest allele, so that SNPs with low frequency alleles are particularly useful as markers of low frequency haplotypes. As particular mutations or polymorphisms associated with certain clinical features, such as adverse or abnormal events, are likely to be of low frequency within the population, low frequency SNPs may be particularly useful in identifying these mutations (for examples see: Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. *Ann Hum Genet* (1998) **62**:481-90, De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, and Gudnason V; and Variation at the von willebrand factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter. *Blood* (1999) **93**:4277-83, Keightley AM, Lam YM, Brady JN, Cameron CL, Lillicrap D).

In a further aspect, the diagnostic methods of the invention are used in the development of new drug therapies which selectively target one or more allelic variants of the Factor X gene. Identification of a link between a particular allelic variant and predisposition to disease development or response to drug therapy may have a significant impact on the design of new drugs. Drugs may be designed to regulate the biological activity of variants implicated in the disease process whilst minimising effects on other variants.

In a further diagnostic aspect of the invention the presence or absence of variant nucleotides is detected by reference to the loss or gain of, optionally engineered, sites recognised by restriction enzymes. In the accompanying Example 2 we provide details of convenient engineered restriction enzyme sites that are lost or gained as a result of a polymorphism of the invention.

According to another aspect of the present invention there is provided a nucleic acid comprising any one of the following polymorphisms:

the nucleic acid of EMBL ACCESSION No. L00394 with T at position 41 as defined by the position in EMBL ACCESSION No. L00394;

the nucleic acid of EMBL ACCESSION No. L00396 with T at position 57 as defined by the position in EMBL ACCESSION No. L00396;

or a complementary strand thereof or an antisense sequence thereto or a fragment thereof of at least 20 bases comprising at least one polymorphism.

Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases.

Novel sequence disclosed herein, may be used in another embodiment of the invention to regulate expression of the gene in cells by the use of antisense constructs. To enable methods of down-regulating expression of the gene of the present invention in mammalian cells, an example antisense expression construct can be readily constructed for instance using the pREP10 vector (Invitrogen Corporation). Transcripts are expected to inhibit translation of the gene in cells transfected with this type construct. Antisense transcripts are effective for inhibiting translation of the native gene transcript, and capable of inducing the effects (e.g., regulation of tissue physiology) herein described. Oligonucleotides which are complementary to and hybridizable with any portion of novel gene mRNA disclosed herein are contemplated for therapeutic use. U.S. Patent No. 5,639,595, Identification of Novel Drugs and Reagents, issued Jun. 17, 1997, wherein methods of identifying oligonucleotide sequences that display in vivo activity are thoroughly described, is herein incorporated by reference. Expression vectors containing random oligonucleotide sequences derived from previously known polynucleotides are transformed into cells. The cells are then assayed for a phenotype resulting from the desired activity of the oligonucleotide. Once cells with the desired phenotype have been identified, the sequence of the oligonucleotide having the desired activity can be identified. Identification may be accomplished by recovering the vector or by polymerase chain reaction (PCR) amplification and sequencing the region containing the inserted nucleic acid material.nucleotide molecules can be synthesized for antisense therapy. These antisense molecules may be DNA, stable derivatives of DNA such as phosphorothioates or methylphosphonates, RNA, stable derivatives of RNA such as 2'-O-alkylRNA, or other oligonucleotide mimetics. U.S. Patent No. 5,652,355, Hybrid

Oligonucleotide Phosphorothioates, issued July 29, 1997, and U.S. Patent No. 5,652,356, Inverted Chimeric and Hybrid Oligonucleotides, issued July 29, 1997, which describe the synthesis and effect of physiologically-stable antisense molecules, are incorporated by reference. Antisense molecules may be introduced into cells by microinjection, liposome encapsulation or by expression from vectors harboring the antisense sequence.

The invention further provides nucleotide primers which can detect the polymorphisms of the invention.

According to another aspect of the present invention there is provided an allele specific primer capable of detecting a Factor X gene polymorphism

at position 41 in exon 5 of the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00394, and/or

at position 57 in exon 7 in the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00396.

An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for ARMS™ assays. The allele specific primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

An allele specific primer preferably corresponds exactly with the allele to be detected but derivatives thereof are also contemplated wherein about 6-8 of the nucleotides at the 3' terminus correspond with the allele to be detected and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the primer.

Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If required the primer(s) may be labelled to facilitate detection.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a Factor X gene polymorphism

at position 41 in exon 5 of the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00394, and/or

at position 57 in exon 7 in the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00396.

The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection.

According to another aspect of the present invention there is provided a diagnostic kit comprising an allele specific oligonucleotide probe of the invention and/or an allele-specific primer of the invention.

The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase.

In another aspect of the invention, the single nucleotide polymorphisms of this invention may be used as genetic markers in linkage studies. This particularly applies to the polymorphism at exon 7 position 57 because of its informative frequency (see below). The Factor X gene has been mapped to chromosome 13q34 (Bowcock et al, Genomics **16**, 486-496, 1993).

According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a Factor Xa ligand antagonist drug in which the method comprises:

i) diagnosis of a single nucleotide polymorphism in Factor X gene in the human, which diagnosis comprises determining the sequence of the nucleic acid

at position 41 in exon 5 of the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00394, and/or

at position 57 in exon 7 in the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00396.

and determining the status of the human by reference to polymorphism in the Factor X gene; and

ii) administering an effective amount of a Factor Xa ligand antagonist drug.

The term " Factor Xa ligand antagonist drug" includes drugs acting at Factor Xa and/or Factor X but the former is preferred.

Factor Xa ligand antagonist drugs possess activity in the treatment or prevention of a variety of medical disorders where anticoagulant therapy is indicated, for example in the treatment or prevention of thrombotic conditions such as coronary artery and cerebro-vascular disease. Further examples of such medical disorders include various cardiovascular and cerebrovascular conditions such as myocardial infarction, the formation of atherosclerotic plaques, venous or arterial thrombosis, coagulation syndromes, vascular injury including reocclusion and restenosis following angioplasty and coronary artery bypass surgery, thrombus formation after the application of blood vessel operative techniques or after general surgery such as hip replacement surgery, the introduction of artificial heart valves or on the recirculation of blood, cerebral infarction, cerebral thrombosis, stroke, cerebral embolism, pulmonary embolism, ischaemia and angina (including unstable angina).

Preferably determination of the status of the human is clinically useful. Examples of clinical usefulness include deciding which antagonist drug or drugs to administer and/or in deciding on the effective amount of the drug or drugs.

Inhibitors of Factor Xa have been disclosed in the following publications: European patent application EP 540051 A, Daiichi; WO98/21188, Zeneca Ltd and WO96/10022, Zeneca Ltd.

According to another aspect of the present invention there is provided use of a Factor Xa ligand antagonist drug in preparation of a medicament for treating a Factor Xa and/or Factor X-mediated disease in a human diagnosed as having a single nucleotide polymorphism at position 41 in exon 5 of the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00394, and/or

at position 57 in exon 7 in the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00396.

According to another aspect of the present invention there is provided a pharmaceutical pack comprising a Factor Xa-ligand antagonist drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism

at position 41 in exon 5 of the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00394, and/or

at position 57 in exon 7 in the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00396.

According to another aspect of the present invention there is provided a computer readable medium comprising at least one novel polynucleotide sequence of the invention stored on the medium. The computer readable medium may be used, for example, in homology searching, mapping, haplotyping, genotyping or pharmacogenetic analysis or any other bioinformatic analysis. The reader is referred to Bioinformatics, A practical guide to the analysis of genes and proteins, Edited by A D Baxevanis & B F F Ouellette, John Wiley & Sons, 1988. Any computer readable medium may be used, for example, compact disk, tape, floppy disk, hard drive or computer chips.

The polynucleotide sequences of the invention, or parts thereof, particularly those relating to and identifying the single nucleotide polymorphisms identified herein represent a valuable information source, for example, to characterise individuals in terms of haplotype and other sub-groupings, such as investigation of susceptibility to treatment with particular drugs. These approaches are most easily facilitated by storing the sequence information in a computer readable medium and then using the information in standard bioinformatics programs or to search sequence databases using state of the art searching tools such as "GCC". Thus, the polynucleotide sequences of the invention are particularly useful as components in databases useful for sequence identity and other search analyses. As used herein, storage of the sequence information in a computer readable medium and use in sequence databases in relation to 'polynucleotide or polynucleotide sequence of the invention' covers any detectable chemical or physical characteristic of a polynucleotide of the invention that may be reduced to, converted into or stored in a tangible medium, such as a computer disk, preferably in a computer readable form. For example, chromatographic scan data or peak data, photographic scan or peak data, mass spectrographic data, sequence gel (or other) data.

The invention provides a computer readable medium having stored thereon one or a more polynucleotide sequences of the invention. For example, a computer readable medium is provided comprising and having stored thereon a member selected from the group consisting of: a polynucleotide comprising the sequence of a polynucleotide of the invention,

a polynucleotide consisting of a polynucleotide of the invention, a polynucleotide which comprises part of a polynucleotide of the invention, which part includes at least one of the polymorphisms of the invention, a set of polynucleotide sequences wherein the set includes at least one polynucleotide sequence of the invention, a data set comprising or consisting of a polynucleotide sequence of the invention or a part thereof comprising at least one of the polymorphisms identified herein.

A computer based method is also provided for performing sequence identification, said method comprising the steps of providing a polynucleotide sequence comprising a polymorphism of the invention in a computer readable medium; and comparing said polymorphism containing polynucleotide sequence to at least one other polynucleotide or polypeptide sequence to identify identity (homology), i.e. screen for the presence of a polymorphism.

The invention will now be illustrated but not limited by reference to the following Examples. All temperatures are in degrees Celsius.

In the Examples below, unless otherwise stated, the following methodology and materials have been applied.

AMPLITAQ™, available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

Electropherograms were obtained in a standard manner: data was collected by ABI377 data collection software and the wave form generated by ABI Prism sequencing analysis (2.1.2).

Example 1

Identification of Polymorphisms

1. Methods

DNA Preparation

DNA was prepared from frozen blood samples collected in EDTA following protocol I (Molecular Cloning: A Laboratory Manual, p392, Sambrook, Fritsch and Maniatis, 2nd Edition, Cold Spring Harbor Press, 1989) with the following modifications. The thawed

blood was diluted in an equal volume of standard saline citrate instead of phosphate buffered saline to remove lysed red blood cells. Samples were extracted with phenol, then phenol/chloroform and then chloroform rather than with three phenol extractions. The DNA was dissolved in deionised water.

Template Preparation

Exons 5 and 7 were amplified from genomic DNA by PCR. Templates were prepared using the oligonucleotide primers described below.

Exon 5 was amplified in a two step PCR reaction with an annealing temperature of 68° and denaturation temperature of 94°. Exon 7 was amplified in a three step PCR reaction with an annealing temperature of 64°, extension temperature of 72° and denaturation temperature of 94°. Each step was 1 minute. Both reactions were carried out in 1.0mM $MgCl_2$ buffer.

For analysis generally 50 ng of genomic DNA was used in each reaction and subjected to 35 cycles of PCR.

Fragment	Forward Oligo 5'-3'	Reverse Oligo
Exon 5	ccagcctccatttctccagctg SEQ ID NO.1	ctggcaggtaacagtgacacca SEQ ID NO.2
Exon 7	caggcaacacctgtctacctg SEQ ID NO.3	gcaccgtcactgtctacttttttca SEQ ID NO.4

Forward oligos were modified by the addition of M13 forward sequence to the 5' end for use in dye-primer sequencing.

Dye Primer Sequencing

Dye-primer sequencing using M13 forward primer was as described in the ABI protocol P/N 402114 for the ABI Prism™ dye primer cycle sequencing core kit with "AmpliTaq FS"™ DNA polymerase, modified in that the annealing temperature was 45° and DMSO was added to the cycle sequencing mix to a final concentration of 5 %.

The extension reactions for each base were pooled, ethanol/sodium acetate precipitated, washed and resuspended in formamide loading buffer.

4.25 % Acrylamide gels were run on an automated sequencer (ABI 377, Applied Biosystems).

2. Results

Novel Polymorphisms

EMBL Sequence	Position	Published	Variant	RFLP	Frequency
L00394	41	C	T	eng Nco I	1/54
L00396	57	C	T	eng Spe I	39/48

Frequency is the allele frequency of the variant allele in control subjects.

"eng"' = engineered RFLP

Example 2

Engineered restriction site primers for detection of polymorphisms

Standard methodology can be used to detect the polymorphism at position 41 (as defined by the position in EMBL ACCESSION NO L00394) and the polymorphism at position 57 (as defined by the position in EMBL ACCESSION NO. L00396) based on the materials set out below using a cDNA template.

EMBL Sequence	**Position**	**Diagnostic Fragment**	**Forward Oligo**	**Reverse Oligo**
L00394	41	17-156	17-40 Nco I	126-156
L00396	57	1-81	1-21	58-81 Spe I

Primer Sequence 5′-3′

17-40 Nco I ACGGAAGCTCTGCAGCCTGGACCA SEQ ID NO.5

58-81 Spe I TAGGATGTAGAACTCGCTCAGACT SEQ ID NO.6

T at position 41 generates an engineered Nco I site in the diagnostic fragment 17-156 described above. T at 57 generates an engineered Spe I site in the diagnostic fragment 1-81 as described above.

Sequence Listing Free Text

SEQ ID NO.1 <223>Description of Artificial Sequence: exon 5 forward primer

SEQ ID NO.2 <223>Description of Artificial Sequence: exon 5 reverse primer

SEQ ID NO.3 <223>Description of Artificial Sequence: exon 7 forward primer

SEQ ID NO.4 <223>Description of Artificial Sequence: exon 7 reverse primer

SEQ ID NO.5 <223>Description of Artificial Sequence: 17-40 Nco I primer

SEQ ID NO.6 <223>Description of Artificial Sequence: 58-81 Spe I primer

CLAIMS

1. A method for the diagnosis of a single nucleotide polymorphism in a Factor X gene in a human, which method comprises determining the sequence of the nucleic acid of the human at position 41 in exon 5 of the Factor X gene as defined by the position in EMBL ACCESSION NO. L00394, and/or at position 57 in exon 7 of the Factor X gene as defined by the position in EMBL ACCESSION NO. L00396, and determining the status of the human by reference to polymorphism in the Factor X gene.

2. A method for diagnosis according to claim 1 in which the single nucleotide polymorphism is further defined as:
the single nucleotide polymorphism at exon 5 position 41 is presence of C and/or T;
the single nucleotide polymorphism at exon 7 position 57 is presence of C and/or T.

3. A method for diagnosis according to claim 1 or 2 in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length polymorphism.

4. Use of a method according to any of claims 1 - 3 for predicting the clinical response to a therapeutic compound, or for determining the therapeutic dose of a compound, in the treatment of Factor X- and/or Factor Xa- mediated disease.

5. Use of a method according to any of claims 1 - 3 for assessing the predisposition of an individual to diseases mediated by Factor X and/or Factor Xa.

6. A nucleic acid comprising any one of the following polymorphisms: the nucleic acid of EMBL ACCESSION NO. L00394 with T at position 41 as defined by the position in EMBL ACCESSION NO. L00394; and/or the nucleic acid of EMBL ACCESSION NO. L00396 with T at position 57 as defined by the position in EMBL ACCESSION NO. L00396; or a complementary strand thereof or an antisense sequence thereto or a fragment thereof of at least 20 bases comprising at least one polymorphism.

7. An allele-specific primer capable of detecting a Factor X gene polymorphism at position 41 in exon 5 of the Factor X gene as defined by the position in EMBL ACCESSION NO. L00394 and/or at position 57 in exon 7 in the Factor X gene as defined by the position in EMBL ACCESSION NO. L00396.

8. An allele-specific oligonucleotide probe capable of detecting a Factor X gene polymorphism at position 41 in exon 5 of the Factor X gene as defined by the position in EMBL ACCESSION NO. L00394 and/or at position 57 in exon 7 in the Factor X gene as defined by the position in EMBL ACCESSION NO. L00396.

9. A diagnostic kit comprising an allele-specific primer as defined in claim 7 or an allele-specific oligonucleotide probe as defined in claim 8.

10. A method of treating a human in need of treatment with a Factor Xa ligand antagonist drug in which the method comprises:

(i) diagnosis of a single nucleotide polymorphism in the Factor X gene in the human, which diagnosis comprises determining the sequence of the nucleic acid at position 41 in exon 5 of the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00394, and/or at position 57 in exon 7 in the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00396, and determining the status of the human by reference to polymorphism in the Factor X gene;

and

(ii) administering an effective amount of a Factor Xa ligand antagonist drug.

11. Use of a Factor Xa ligand antagonist drug in the preparation of a medicament for treating a Factor Xa and/or Factor X mediated disease in a human diagnosed as having a single nucleotide polymorphism at position 41 in exon 5 of the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00394, and/or at position 57 in exon 7 in the Factor X gene as defined by the positions in EMBL ACCESSION NO. L00396.

12. A computer readable medium comprising at least one nucleic acid sequence as defined in claim 6 stored on the medium.

SEQUENCE LISTING

<110> ZENECA Limited

<120> CHEMICAL COMPOUNDS

<130> CJC/PHM 70433/WO

<140> 9826747.9

<141> 1999-12-05

<150> GB 9826747.9

<151> 1998-12-05

<160> 6

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<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Exon 5 forward primer

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<210> 2

<211> 22

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Exon 5 reverse primer

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<220>
<223> Description of Artificial Sequence: Exon 7 forward primer

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<210> 4
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<220>
<223> Description of Artificial Sequence: Exon 7 reverse primer

<400> 4
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<223> Description of Artificial Sequence: 17-40 NCO I primer

<400> 5
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<210> 6

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: 58-81 Spe I primer

<400> 6

taggatgtag aactcgctca gact 24

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03973

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 A61P43/00 A61K31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	COOPER DN ET AL: "Inherited Factor X deficiency: Molecular genetics and pathophysiology" THROMBOSIS AND HAEMOSTASIS, vol. 78, no. 1, July 1997 (1997-07), pages 161-172, XP000890130 page 166; table 1 —	1-5,7-11
A	MIYATA T ET AL: "Factor X Nagoya 1 and Nagoya 2: a CRM- defiency and a dysfunctional CRM+ Factor X defiency characterized by substitution of Arg306 by Cys and of Gly366 by Ser, respectively." THROMBOSIS AND HAEMOSTASIS, vol. 79, no. 3, March 1998 (1998-03), pages 486-90, XP000889942 the whole document —	1-5,7-11
	-/—	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 March 2000	21/03/2000

Name and mailing address of the ISA	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03973

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCHAFER AJ ET AL: "DNA variation and the future of human genetics" NATURE BIOLOGY, vol. 16, January 1998 (1998-01), XP000890128 the whole document	1
A	WO 98 38318 A (FALKNER FALKO GUENTER ;HIMMELSPACH MICHELE (AT); EIBL JOHANN (AT);) 3 September 1998 (1998-09-03) see SEQ ID No 43, where in bp position 793, an A is indicated in place of a C found in EMBL ACC No L00396, corresponding to nucleic acid sequence of Exon 7.	1,2

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/03973

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 10 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter...l Application No
PCT/GB 99/03973

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9838318 A	03-09-1998	AT 405517 B	27-09-1999
		AT 33697 A	15-01-1999
		AU 6080898 A	18-09-1998
		NO 994136 A	27-10-1999